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Project Completion
Report No. 712431

1980

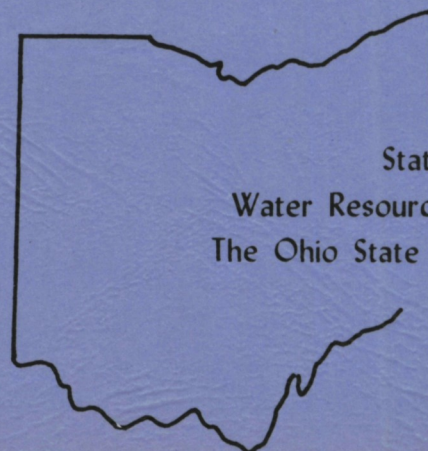
**BIOCLEANSING WITH
AQUATIC WEEDS:
A MEANS OF REMOVING
ASBESTIFORM FIBERS
FROM WATER**

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Contract No.
A-057-OHIO



State of Ohio
Water Resources Center
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A MEANS OF REMOVING ASBESTIFORM FIBERS FROM WATER

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December, 1980

This study was supported in part by the
Office of Water Research and Technology
U.S. Department of the Interior under
Project A-057-OHIO

Introduction

Industrial exposure to asbestos fibers has long been associated with cancer of the lung and peritoneum as well as asbestosis (Brodeau, 1972). Recently, asbestos fibers have been identified in such diverse media as talc-coated rice (Cunningham, 1973) and drinking water (Cunningham, Pontefract, 1971; Cook et al., 1974; Kay, 1974; American Water Works Association, (1974). Experimental studies in animals indicate that asbestos injected directly into the stomach is concentrated two days later in the omentum surrounding the large intestines. Asbestos was also found in the brain and the blood stream (Blejer, 1973). A normally rare type of cancer in humans, mesothelioma, has been produced in animals by intrapleural inoculation of various types of asbestos (Wagner, 1973). While ingestion of these fibers in drinking water has not to date been established as a human health hazard, the possibility of a potential health hazard should not be ignored.

Little information is available on the effects of asbestos ingestion in animals or man. The dynamics of passage of material through the lumen and the wall of the gastrointestinal tract is very complex. It has been theorized that the fibers pass through the gut wall, reach the peritoneum and are widely disseminated. This, however, has not been actually demonstrated in man, and animal experimental results have varied. The appearance of fibers in the blood after injection into the stomach has been shown, and their later presence in various organs has been demonstrated (Pontefract, 1974).

Some investigations have demonstrated stimulation of DNA synthesis, as evidenced by increased incorporation of tritiated thymidine, in the gastrointestinal tract of rats following single lavaging with asbestos (Amacher et al., 1974, 1975). Studies with monkeys have demonstrated a similar increase

in DNA synthesis (Epstein and Varnes, 1976). Increased synthesis of pancreatic DNA in primates and similar effects in the gastrointestinal tracts of rats at relatively short intervals following the ingestion of asbestos may reflect DNA replication following asbestos-induced cytotoxicity or a direct stimulation of DNA replication.

In the most recent studies rats were fed diets containing 0.5 mg or 50 mg of chrysotile asbestos each day for 1 week for 14 months and tissues of the gastrointestinal tract were examined by light and electron microscopy (Jacobs et al., 1978). At the light microscope level the esophagus, stomach and caecum in treated animals appeared unaffected, whereas, accumulation of cellular debris and Alcian blue-positive material was apparent in the ileal, rectal, and colonic lumen. Electron microscope examination of the colon and ileum of rats ingesting 50 mg chrysotile/day for 14 months confirmed findings and indicated changes in the mucosal lining cells of the ileum which were consistent with a mineral-induced cytotoxicity.

The presence of amphibole mineral fibers in the waters of Lake Superior was established in 1969. Since many cities obtain water from Lake Superior, asbestos fibers have been found in their drinking water supplies. The City of Duluth, Minnesota has reported fiber counts ranging from 1 million to 100 million fibers per liter in its drinking water. Lake Michigan has also been determined to contain asbestos fibers. Chicago has reported fiber counts in drinking water as high as .5 million fibers per liter.

The extremely high fiber counts reported in many locations aroused much public interest, particularly in Duluth, Minnesota. In 1974 a project began there to design and construct a water treatment plant capable of removing asbestos fibers. The plant was built at a cost of approximately \$6 million and is now operational with questionable results.

Since the completion of the Duluth, Minnesota water treatment plant many cities have been adopting projects which will help aid in the removal of asbestos fibers from drinking water supplies. The most common approaches include coagulation; flocculation with either ferric chloride or alum plus a polyelectrolyte, coagulation of added bentonite clay with polyelectrolyte, diatomaceous earth filtration and simple sand filtration. These procedures will remove a certain amount of the larger fibers, however, a major portion of the smaller fibers remain.

Recently, researchers have discovered that some aquatic weeds can scavenge inorganic, and some organic compounds from water. Effluent removed by the plants is stripped of its pollutants. The plant culture unite clean water so rapidly and effectively that they are now seriously being considered for use as a "final polish" in sewage treatment. It has also recently been shown by this laboratory that certain root systems grown in culture are capable of internal and external accumulation of asbestos. The primary objective of this study was to find a suitable aquatic plant for the removal of asbestos fibers from water supplies. The discovery of such a system would be a major advancement in water purification technology and could virtually solve the problems of asbestos contamination in municipal water supplies.

Methods

In order to determine the efficiency of asbestos fiber removal by aquatic plants, each was grown in a small (35 liters) and a large (200 liters) capacity aquarium. The aquaria were filled with water which has been filtered with a 0.2 μm nucleopore filter. The fiber concentration in the 35 liter aquaria was adjusted to approximately 1.50×10^6 fibers/liter and that in the 200 liter aquaria to 7.5×10^5 fibers/liter using equal parts of chrysotile and tremolite asbestos. Chrysotile and tremolite were chosen because they are representative of both the serpentine and amphibole classes of asbestos.

Approximately 200 grams (wet weight) and 700 grams of plants were introduced into the small and large aquaria respectively. This provides a fiber to plant ratio of 2.63×10^5 fibers/gram in the 35 liter aquaria and 2.14×10^5 fibers/gram in the 200 liter aquaria. Two air stones in the center of the aquaria provided circulation of the water to allow for maximum contact between the asbestos fibers and the plants. However, even with the circulation, some of the larger fibers probably settled to the bottom of the tanks. This was not considered a problem because those fibers would easily be removed from municipal water supplies by conventional flocculation and sedimentation.

Water samples were taken from the aquaria on a weekly basis for ten weeks to determine the fiber count in relation to time. The water samples were prepared by first passing them through a 0.2 μm polycarbonate membrane filter which had previously been coated with a thin film (200A) of evaporated carbon. The filtration was performed under a controlled filtered atmosphere hood, which had a laminar air flow to reduce the possibility of

airborne contamination. The filter with the asbestos particles was then coated with another thin film of evaporated carbon. The filter was then cut into squares approximately $(3 \text{ mm})^2$ and squares were placed carbon side down on blank transmission electron microscope grids. A single drop of chloroform was placed on each filter square and the squares were then placed in a petri dish with several filter discs saturated with chloroform. When all the polycarbonate filter had dissolved away the specimens were observed using a Zeiss EM-9s Electron Microscope.

Water samples were taken from tanks containing plants and asbestos as well as from the controls which consisted of filtered water with no asbestos and no plants, filtered water with asbestos only, and filtered water with plants only. Twenty separate grids were prepared for each water sample. Enumeration was done using standard procedures of analysis for waterborne asbestos (Hallenbeck et al., 1977). Some of the plant material exposed to asbestos was observed using electron microscopy to determine the type of interaction between the plants and the asbestos. After ten weeks of growth in the presence of asbestos, the plants were removed from the tanks and cut into lengths approximately 5 mm long. These were placed in a formaldehyde-glutaraldehyde fixative for $4\frac{1}{2}$ hours at 25°C (Karnovsky, 1965).

After two rinses in 0.1 m Sorensens phosphate buffer (pH 7.35), the tissue was postfixed in 1.33% osmium tetroxide buffered with s-collidine (pH 7.40) for 2 hours at 4°C . Following two rinses in 0.2 s-collidine (pH 7.40), the material was placed in 1% uranyl acetate in 10% ethanol for 20 minutes at 25°C . The tissue was dehydrated for 30 minutes in each of the following: 50% ethanol, 70% ethanol, 90% ethanol. twice in 95% ethanol, and twice in

100% ethanol. Two 30-minute rinses in propylene oxide removed the ethanol before infiltration with Epon 812. After polymerization, thin sections were cut on a Reichart "Om U2" ultramicrotome with a DuPont diamond knife.

RESULTS

The studies were conducted with *Elodia*, *Seirpus*, *Sagittaria*, and common pondweed. Five repetitions were performed with each plants type in both the 35 liter and 200 liter aquaria.

Elodea recorded the greatest overall reduction at the end of ten weeks. In the 35 liter aquaria there were reductions up to 48%. During the first week there were reductions as great as 15% and by the end of four weeks there were reductions of as much as 46%. As the analysis continued the fiber count was not reduced significantly below that observed on the fourth week.

Elodea grown in the 200 liter aquaria recorded reductions of as much as 65%. The reductions during the first week were very much similar to those recorded in the smaller aquaria. However, in the larger aquaria continuous reductions were noted up until the ninth week, after which time no significant reductions were recorded.

Common pondweed demonstrated the second greatest ability to scavenge asbestos. During the first week the maximum reduction in fiber concentration was 8% in the 35 liter aquaria. Maximum overall reduction was recorded in most cases during the sixth week. The greatest reduction recorded was 28%. In the larger aquaria reductions of as much as 52% were recorded. In the 200 liter aquaria significant reductions proceeded until the eighth week. After that time only minor decreases in fiber concentration were recorded.

The greatest first week reduction for *Sagittaria* was 4% in the small aquaria and 5% in the large aquaria. The greatest reduction observed in the 35 liter aquaria was 26% and that in the 200 liter aquaria was 34%. The patterns of reduction were very much similar to those of *elodea* and pondweed.

Scirpus produced first week reductions of as much as 9% in the 35 liter aquaria with final reductions of as much as 25%. In the 200 liter aquaria first week reduction as high as 12% were recorded with overall reductions of as much as 39%.

Figure 1 compiles all the results discussed above in tabular form. The average weekly reduction is shown for each plant in both the 35 liter and the 200 liter aquaria. Below the average reduction is the standard deviation.

The control tanks containing no plants and no asbestos and the ones containing plants and no asbestos were also tested weekly. There were no fibers detected in any of these control tanks for the duration of the tests. The control **tanks** containing asbestos and no plants were also tested weekly. The fiber count remained approximately constant with a standard deviation of 5%.

Material was taken from each type of plant and observed in an electron microscope to determine the type of interaction between the plants and the asbestos fibers. The results of these observations would indicate that the fibers do accumulate externally on the plants. There was no evidence to indicate that the fibers also accumulate internally in the plants. The external bond was very strong and could possibly be due to an electrochemical interaction between the cell wall of the plant and the asbestos fibers.

Plant Type	Aquar. Size (Liters)		1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week	9th Week	10th Week
Elodea	35	Average reduction Standard deviation	9.0% 4.1	15.0% 4.9	26.0% 6.7	35.4% 9.2	35.8% 9.5	35.6% 8.8	35.0% 7.0	37.0% 7.7	37.6% 7.1	35.6% 7.3
Elodea	200	Average reduction Standard deviation	10.2% 2.6	16.6% 6.3	22.6% 6.7	31.8% 7.3	36.2% 9.0	44.2% 10.0	48.0% 11.3	49.8% 10.6	51.2% 10.8	50.6% 11.2
Pondweed	35	Average reduction Standard deviation	5.4% 2.1	8.0% 25.	12.4% 3.0	18.0% 3.3	22.0% 3.3	23.2% 3.6	23.2% 3.9	24.4% 2.7	23.8% 2.5	23.8% 2.8
Pondweed	200	Average reduction Standard deviation	6.2% 1.9	12.0% 2.8	14.2% 9.0	18.4% 11.1	30.2% 6.9	35.2% 7.8	38.6% 5.1	41.2% 5.1	42.0% 5.7	43.2% 5.5
Sagittaria	35	Average reduction Standard deviation	3.2% 0.8	7.6% 1.7	11.4% 1.5	14.4% 2.2	18.2% 4.1	19.0% 4.1	19.2% 3.1	19.4% 3.5	18.6% 1.9	19.2% 2.3
6 Sagittaria	200	Average reduction Standard deviation	3.4% 1.1	6.2% 1.8	9.4% 2.1	13.0% 2.8	16.4% 3.4	21.6% 5.5	24.2% 5.3	25.8% 5.8	25.4% 4.8	26.8% 4.8
Scirpus	35	Average reduction Standard deviation	3.8% 3.1	7.4% 1.1	10.4% 2.6	13.6% 2.7	15.6% 2.4	18.6% 2.3	20.4% 1.9	22.4% 2.9	20.6% 1.3	21.2% 3.7
Scirpus	200	Average reduction Standard deviation	7.0% 3.5	10.8% 2.8	13.0% 4.6	17.8% 4.8	21.0% 6.6	24.8% 4.1	27.4% 5.1	29.2% 6.4	32.6% 5.0	30.6% 2.7

Figure 1: Average reduction in asbestos fiber concentration measured in percentage. Standard deviation is also listed.

DISCUSSION

All types of plants tested removed asbestos fibers to some degree. By calculating the number of fibers removed per gram of plant material a comparison of the ability of the plants to remove asbestos can be made. Elodea clearly removes the greatest number of fibers per gram of plant material. In the 35 liter aquaria an average of 9.35×10^4 fibers were removed per gram of elodea. In the larger tanks 1.08×10^5 fibers were removed. Pondweed removed an average of 6.25×10^4 and 9.26×10^4 fibers in the 35 and 200 liter tanks, respectively. Scirpus removed 5.57×10^4 fibers in the 35 liter aquaria and 6.56×10^4 fibers in the 200 liter aquaria while Sagittaria removed 5.04×10^4 fibers from the small tanks and 5.74×10^4 fibers from the large tanks. These numbers suggest that the ability of a particular plant to remove asbestos is a set quantity. When that quantity is reached the plant becomes saturated. This is evidenced by the relatively small variation in the quantity of fibers removed per gram of plant material in the large and small tanks. The number of fibers which can be removed per gram of any plant tested appears to be a set value for that plant no matter what the concentration of asbestos fibers.

From the data collected it can be concluded that the ability of a plant to scavenge asbestos is directly proportional to the surface area of the plant. Elodea, with its many leaves, has a far greater surface area than the other plants tested. Pondweed has the second greatest surface area, followed by Scirpus and Sagittaria which have a relatively small surface area. The relationship to surface area is further supported by the electron micro-

scope studies which show that the fibers only accumulate externally. Elodea also has a greater rate of growth which could increase its amount in the aquaria faster than the others.

CONCLUSION

It appears that aquatic plants are capable of removing asbestos fibers from their surrounding environment. Each plant type has a characteristic number for the quantity of asbestos that can be removed per gram of the plant. This number is directly proportional to the surface area of the plant. Electron microscopy reveals that the asbestos fibers accumulate externally on the plant surface, possibly through an electrochemical reaction.

The results of this study suggest that plants should be left in reservoirs to scavenge asbestos fibers. Further studies need to be performed using outdoor holding ponds to determine the feasibility of incorporating this type of filtration system. If the results of those studies parallel the laboratory studies several holding ponds could be incorporated at water treatment centers. After allowing the water to stand in ponds for several weeks, the ponds could be drained and the plants removed and disposed of. This means of biocleansing could eliminate asbestos from drinking water at a minimal cost.

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